ONR FINAL REPORT

GRANT #: N00014-93-10229

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GRANT TITLE: Formation of Calcite Biocrystals; Structure and Formation of Matrix Glycoproteins

REPORTING PERIOD: Final

AWARD PERIOD: 03/01/93 - 02/28/97

I. Studies in the Distribution of Protein in the Sea Urchin Spicule

The presence of proteins associated with the $CaCO_3$-containing biocrystals found in a wide variety of marine organisms is well established. In these organisms, including the primitive skeleton (spicule) of the sea urchin embryo, the structural and functional role of these proteins either in the biomineralization process or in control of the structural features of the biocrystals is unclear. Recently, one of the matrix proteins of the sea urchin spicule, SM30, has been shown to contain a carbohydrate chain (the 1223 epitope) that has been implicated in the process whereby Ca^{2+} is deposited as $CaCO_3$. Because an understanding of the localization of this protein, as well as other proteins found within the spicule, is central to understanding their function, we undertook to develop methods to localize spicule matrix proteins in intact spicules, using immunogold techniques and scanning electron microscopy. Gold particles indicative of this matrix glycoprotein could not be detected on the surface of spicules that had been isolated from embryo homogenates and treated with alkaline hypochlorite to remove any associated membranous material. However, when isolated spicules were etched for 2 min with dilute acetic acid (10 mM) to expose more internal regions of the crystal, SM30 and perhaps other proteins bearing the 1223 carbohydrate epitope were detected in the calcite matrix. These results, indicating that these two antigens are widely distributed in the spicule, suggest that this technique should be applicable to any matrix protein for which antibodies are available.

II. Studies on the Role of Sea Urchin Done Morphogenetic Protein (suBMP) in Differentiation of Spicule Forming Primary Mesenchymal Cells

On going work in our laboratory has shown that suBMP, a bone morphogenetic protein 1 family member, is the homolog of the Drosophila embryonic patterning gene tolloid. Resequencing of the 3' end of the suBMP cDNAs has revealed an extended open reading from with high homology to human and murin tolloid homologs. Recent studies in the laboratories of Darwin Prockop, Daniel Greenspan, and Effrat Kessler, have demonstrated that both human bone morphogenetic protein 1 and human tolloid act as procollagen C-terminal proteinases, releasing the C-terminal propeptide from procollagen. This proteolytic processing event is necessary for the deposition of collagen fibrils. We have been able to demonstrate the existence of a procollagen C-terminal proteinase (PCP) activity in *S. purpuratus* extracts containing suBMP. This PCP activity is heat labile, and

demonstrates time dependent cleavage. Current studies are underway to determine if recombinant suBMP has PCP activity.

Previous studies in our lab and others have demonstrated that appropriate collagen processing is necessary for gastrulation and spiculogenesis to occur in the developing sea urchin embryo, as well as for calcium carbonate deposition into growing spicules in primary mesenchyme cell culture. Disruption of collagen hydroxylation or crosslinking blocks spicule growth. SuBMP may also play an important role in this process. By removing the C-terminal propeptide from triple-helical procollagen, the collagen triple helix is rendered insoluble, and can be deposited into growing collagen fibrils. If suBMP is responsible for the PCP activity in *S. purpuratus*, then its function will be essential for collagen deposition and therefore sea urchin development.

III. Publications Supported by the ONR Grant:

1. Cho, J.W., Partin, J.S. and Lennarz, W.J. (1996), A Technique for Detecting Matrix Proteins in the Crystalline Spicule of the Sea Urchin Embryo. Proc. Natl. Acad. Sci. 93, 1281-1286.

2. Brown, Martin, F., Partin, Jacqueline S., Killian, Christopher E. and Lennarz, William J. (1995), Spiculogenesis in the Sea Urchin Embryo: Studies on the SM30 Spicule Matrix Protein. Develop. Growth and Differ. 37, 69-78.

3. Lennarz, William J. (1994), Fertilization in Sea Urchins: How many Different Molecules are Involved in Gamete Interaction and Fusion. Zygote 2, 1-4.

4. Hwang, Sheng-Ping L., Partin, Jacqueline S. and Lennarz, William J. (1994), Characterization of a Homolog of Human Bone Morphogenetic Protein 1 in the Embryo of the Sea Urchin, *S. purpuratus*. Development 120, 559-568.

5. Hwang, Sheng-Ping L. and Lennarz, William J. (1993), Studies on the Cellular Pathway Involved in Assembly of the Embryonic Sea Urchin Spicule. Exp. Cell Res. 205, 383-387.

REPORT OF INVENTIONS AND SUBCONTRACTS

Pursuant to "Patent Rights" Contract Clause) See Instructions on Reverse Side.)

FORM APPROVED
OMB NO. 0704 0016

1a. Name of Contractor/ Subcontractor	c. Contract Number	2a. Name of Government Prime Contractor	c. Contract Number	3. Type of Report (check one)
The Research Foundation of SUNY	N000149310229	Office of Naval Research		☐ Interim ☒ Final
b. Address (include Zip Code) Office of Sponsored Programs SUNY at Stony Brook Stony Brook New York 11794-3366	d. Award Date (yymmdd) 93/03/01	b. Address (include Zip Code)	d. Award Date (yymmdd)	4. Reporting Period (yymmdd) From: 93/03/01 To: 97/02/28

SECTION I - SUBJECT INVENTIONS

5. "SUBJECT INVENTIONS" REQUIRED TO BE REPORTED BY CONTRACTOR/SUBCONTRACTOR (If "None", so state)

a. Name of Inventor(s) (Last, First, M.I.)	b. Title of Invention(s)	c. Disclosure No. Patent application Serial No. or Patent No.	d Election to File Patent Applications				e. Confirmatory Instrument or Assignment Forwarded To Contracting Officer	
			United States		Foreign			
			Yes	No	Yes	No	Yes	No
	None							

f. Employer of Inventor(s) Not Employed by Contractor/Subcontractor		g. Elected Foreign Countries in which a Patent Application will be Filed	
I. Name of Inventor	I. Name of Inventor	I. Title of Invention	II. Foreign Countries of Patent Application
II. Name of Employer	II. Name of Employer		
III. Address of Employer (include zip code)	III. Address of Employer (include zip code)		

SECTION II- SUBCONTRACTS (Containing a "Patent Rights" clause)

6. Subcontracts Awarded by Contractor/Subcontractor (if "None", so state)

a. Name of Subcontractor(s)	b. Address (include Zip Code)	c. Subcontract No.(s)	d. DAR "Patent Rights"		e. Description of Work to be Performed Under Subcontracts	f. Subcontracts Dates (yymmdd)	
			Clause No.	Date (yymmdd)		Award	Estimated Completion
None							

SECTION III - CERTIFICATION

7. Certification of Report by Contractor/Subcontractor (Not Required if ☐ Small Business or ☐ Non-Profit Organization.) (Check Appropriate Box.)

a. Name of Authorized Contractor/Subcontractor Official (Last,First,M.I.) Petersen, John C.	c. I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions", that such procedures have been followed and that all "Subject Inventions" have been reported.
b. Title Director of Technology Licensing	John Petersen — Signature of Authorized Contractor/Subcontractor Official; 97/10/14 — Date (yymmdd)

DD Form 882
82 Oct

Edition of 1 Oct 75 is Obsolete